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Journal of
**Agricultural
and Food
Chemistry®**

Inhibition of Enzymatic Browning in Fresh Fruit and Vegetable Juices by Soluble and Insoluble Forms of β -Cyclodextrin Alone or in Combination with Phosphates

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Soluble and insoluble forms of β -cyclodextrin (β -CD) can be used to inhibit enzymatic browning of fresh raw apple, pear, white grape, and celery juice. The anti-browning effects of soluble CDs were concentration-dependent. Concentrations (1–1.5% w/v) of soluble (un-derivatized, hydroxyethyl, or maltosyl) β -CD substantially inhibited browning of Granny Smith apple juice held at room temperature for several hours while untreated juice browned in minutes. Higher concentrations (4–10%) of soluble β -CD derivatives completely inhibited the browning of apple juice held for 1 day under these conditions. The effectiveness of β -CD was greatly enhanced by the presence of phosphate-containing compounds. Hence, only 1% levels of soluble β -CD (in the presence of 0.25–0.5% phosphate compound) completely inhibited browning in apple juice held for 1 day at room temperature or for up to 2–3 weeks at 4 °C. Treatment of apple, pear, white grape, and celery juice with an insoluble form of β -CD, either in a batchwise or flow-through process, resulted in juices free of CDs that resisted browning indefinitely.

Keywords: *Enzymatic browning; inhibition; cyclodextrins; vegetable and fruit juice; phosphates; phytic acid*

INTRODUCTION

In recent years, the produce industry has undergone a large and growing shift in marketing from fresh commodities to minimally processed (fresh-cut) fruits and vegetables. As part of this trend, juice processors are showing increased interest in marketing juice products that are similar in sensory characteristics to fresh juice. When raw fruits and vegetables are peeled, cut, sliced, or pressed to produce juice, the resulting products frequently undergo rapid enzyme-catalyzed browning reactions. This browning is the result of processing-induced physical damage to the plant tissue, in which naturally occurring monophenolic compounds mixed with atmospheric oxygen and endogenous polyphenol oxidase (PPO) enzymes are hydroxylated to *o*-diphenols and together with endogenous *o*-diphenols are then oxidized to *o*-quinones. These quinones may condense and react nonenzymatically with other phenolic compounds, amino acids, proteins, and other cellular constituents to produce colored polymers and pigments [see reviews by Vamos-Vigyazo (1981) and Sapers (1993)]. Such browning reactions have an important bearing on food quality and marketability

and are therefore of great importance to the fruit and vegetable processing industry. Until 1986, enzymatic browning in these products could be inhibited commercially by the application of sulfites. However, sulfites have been associated with severe allergy-like reactions in certain populations, and as a result, the Food and Drug Administration (FDA) has limited their use to only a few current applications (Sapers et al., 1993). Because none of the currently used sulfite substitutes are as effective as sulfites per se, there is a need for the development of more effective browning inhibitors.

Numerous inhibitors of enzymatic browning have been identified [see reviews by Vamos-Vigyazo (1981) and Sapers 1993 and they include such diverse entities as ascorbic acid (AA) (Ponting et al., 1972; Janovitz-Klapp et al., 1972) and its derivatives (Sapers et al., 1989), cysteine and cysteine derivatives (Molnar-Perl and Friedman, 1990a,b), honey (Oszmianski and Lee, 1990), 4-hexylresorcinol (McEvily et al., 1991), and proteases (Labuza et al., 1992). At least three different modes of action for inhibitors can be theorized. These include (1) the direct inhibition of the enzyme PPO, (2) the chemical reduction of *o*-quinones back into *o*-diphenolic compounds, and (3) the manipulation or removal of the phenolic substrates of PPO. Most of the inhibitors mentioned above are thought to operate by either the first or second mechanism. We previously published preliminary information (Sapers et al., 1989) on and patented (Hicks et al., 1990) a process for the inhibition of enzymatic browning in fruit and vegetable juices by the third mechanism, which involved the use of cyclodextrins. We later showed (Irwin et al., 1994) that cyclodextrins form a 1:1 inclusion complex with PPO substrates (i.e., chlorogenic acid), thereby protecting the substrate from enzymatic oxidation.

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Table 1. Inhibition of Browning at Room Temperature in Granny Smith Apple Juice by Various Cyclodextrins

treatment	% Inhibition									
	<i>L</i> value			<i>a</i> value			% <i>R</i> ₄₄₀			
	2 h	6 h	25 h	2 h	6 h	25 h	2 h	6 h	25 h	98 h
β -CD										
1% ^a (8.8 mM)	74 (16) ^b	29 (10)	-1 (8)	80 (16)	31 (15)	11 (19)	78 (17)	33 (13)	13 (8)	
1.5% ^c (13.2 mM)	64 (18)	32 (17)	-8 (13)	80 (11)	37 (12)	8 (3)	76 (11)	42 (7)	17 (4)	
maltosyl β -CD										
1% ^d (~7 mM)	76 (14)	33 (10)	0 (9)	76 (18)	30 (13)	2 (15)	79 (18)	38 (14)	12 (12)	
1.5% ^c (~10.5 mM)	74 (4)	48 (9)	6 (7)	100 (5)	56 (17)	9 (3)	70 (5)	47 (8)	16 (4)	
4% ^d (~28 mM)	140 (6)	124 (8)	73 (29)	134 (32)	106 (23)	31 (14)	106 (15)	99 (9)	60 (10)	
hydroxyethyl β -CD										
1% ^c (~8 mM)	72 (10)	31 (8)	12 (9)	82 (7)	42 (3)	25 (3)	64 (8)	22 (6)	6 (6)	
1.5% ^c (~12 mM)	63 (3)	38 (11)	8 (3)	92 (6)	59 (12)	32 (3)	65 (1)	40 (15)	16 (7)	
4% ^c (~36 mM)	114 (3)	105 (2)	30 (4)	104 (2)	97 (3)	53 (5)	102 (3)	98 (2)	41 (6)	
10% ^c (~80 mM)	104 (3)	105 (2)	113 (20)	88 (18)	91 (7)	86 (7)	105 (5)	108 (3)	114 (3)	102 (10)
sodium bisulfite 100 ppm	108 (2)	111 (2)	145 (4)	107 (1)	109 (1)	116 (1)	101 (1)	101 (1)	106 (1)	

^a Fourteen samples used in treatment study. ^b Values in parentheses are standard deviations of the mean. ^c Four samples used in treatment study. ^d Six samples used in treatment study.

In this paper, we now provide new and practical information about the inhibition of enzymatic browning in fruit and vegetable juices by β -cyclodextrin (β -CD) and several of its soluble and insoluble derivatives alone and in combination with several inorganic and organic phosphate derivatives.

MATERIALS AND METHODS

Chemicals. β -Cyclodextrin, hydroxyethyl β -CD, hydroxypropyl- β -CD, and insoluble β -CD polymer were provided by American Maize Products Corporation (Hammond, IN). Two sizes of insoluble β -CD polymer were obtained: 20–60 mesh and 60–100 mesh. Each functioned similarly, so no distinction between the sizes will be made below. These insoluble polymers were prepared by the manufacturer by cross-linking monomeric β -cyclodextrin with epichlorohydrin. Branched (maltosyl-) β -CD was provided by the Hereld Organization (Hamden, CT) and the Ensuiko Sugar Refining Co., Ltd. (Yokohama, Japan). Taterfos (sodium acid pyrophosphate, abbreviated hereafter as SAPP) was a gift from Stauffer Chemical Company, Food Ingredients Division (Westport, CT). Glass H (sodium hexametaphosphate, abbreviated hereafter as SHMP) was a gift from FMC Corporation, Industrial Chemical Group (Philadelphia, PA). Sporix, an acidic polyphosphate, was a gift from International Sourcing, Inc. (South Ridgewood, NJ). Phytic acid (dodecasodium salt hydrate) was purchased from Aldrich Chemical Co., Inc., (Milwaukee, WI). L-Ascorbic acid (AA) was purchased from Sigma Chemical Co. (St. Louis, MO).

Juice Preparation and Treatments. Apples (*Malus domestica* Borkh., cv. Granny Smith) were purchased from local supermarkets and stored at 4 °C until needed. Apples were not peeled, but were cored and sliced prior to juicing in an Acme Supreme Juicerator Model 6001 lined with Whatman No. 1 filter paper. Juice was always collected in a beaker containing enough ascorbic acid to produce a final concentration of 50 ppm AA. This added ascorbic acid (Sapers et al., 1989) delayed the onset of browning until test materials could be mixed thoroughly with the juice. Aliquots of juice were added to beakers containing known quantities of test materials. The juice–test material mixture was stirred gently until test materials were solubilized. A total of 50 ppm AA was also added to pure juice samples used as controls. Other types of juices were similarly made from locally procured Thompson seedless grapes, D'anjou pears, and stalk celery.

For experiments with insoluble cyclodextrin polymers, two different techniques were used; a semicontinuous mode and a batchwise mode. In the former case, nonoptimized experiments were performed as follows: 30 mL of freshly prepared fruit or vegetable juice was eluted through 15 mL of hydrated cyclodextrin polymer that had been slurry packed into a glass column. In batchwise experiments, nonoptimized experiments were performed by adding 15 mL of hydrated polymer to 30

mL of fresh juice, stirring carefully for 10 min, and then removing the polymer by sedimentation and decantation. Juices from both types of β -CD polymer treatments were collected, stored at room temperature, and monitored visually by the investigators for color development.

Color Measurements. Except where indicated, all juice samples were measured for Hunter *L* and *a* values and percent reflectance at 440 nm (% *R*₄₄₀) using a Byk-Gardner Color Machine spectrophotometer (Pacific Scientific, Silver Springs, MD). Measurements were made immediately after test materials were dissolved into juice and at timed intervals thereafter. The method of Sapers et al. (1989) was used to transform measurements into values for % inhibition of browning. An inhibition value of 0% indicates no inhibition of browning whereas an inhibition of 100% indicates complete inhibition of browning. Values over 100% inhibition indicate that the color of the sample has become lighter than that of the fresh juice. Such phenomena can occur through chemical reactions such as bleaching. Inhibition values reported in the tables are the means of multiple samples conducted from different lots of apples on different days. Each sample was run in triplicate.

RESULTS AND DISCUSSION

Effects of Soluble Cyclodextrins Alone. Table 1 contains % inhibition values obtained from experiments designed to test the effect of soluble cyclodextrins in the apple juice model system. Percent inhibition values were calculated using each of the three experimentally derived parameters: Hunter *L* values, *a* values and % *R*₄₄₀. Although the absolute values calculated for % inhibition differs slightly for each parameter used, the relative values are all similar. For ease of the discussion, only the % inhibition values calculated from % *R*₄₄₀ values will be used. This is especially appropriate since % *R*₄₄₀ values correlated best with visual observation.

Because we reported earlier (Sapers et al., 1989; Irwin et al., 1994) that β -CD was more effective than either α - or γ -CD, the present study concentrated only on the former cyclic heptasaccharide which, as expected, did inhibit browning for short periods (Table 1) when used at a 1% wt/vol concentration (8.8 mM). Because of the 1:1 inclusion complex formed between chlorogenic acid (the major PPO substrate in apple) and β -CD (Irwin et al., 1994), increasing the concentration of CD in juice should lead to greater inhibition. However, an additional 50% increase in β -CD did not have a measurable effect. This may be because β -CD has low solubility in aqueous solution. Several more water-soluble derivatives of β -CD were tested however. Maltosyl β -CD is more soluble than underivatized β -CD and had inhibi-

Table 2. Inhibition of Browning at Room Temperature in Granny Smith Apple Juice by Cyclodextrins in Combination with Phosphates

treatment	% inhibition								
	<i>L</i> value			<i>a</i> value			% <i>R</i> ₄₄₀		
	2 h	6 h	25 h	2 h	6 h	25 h	2 h	6 h	25 h
0.5% SAPP ^a	36 (1) ^b	4 (1)	0 (0)	54 (3)	18 (2)	22 (1)	40 (5)	6 (3)	6 (7)
0.5% SHMP ^a	53	13 (10)	-25 (8)	42	-7 (14)	-26 (19)	53	26 (7)	18 (6)
0.25% Sporix ^c	74 (11)	49 (18)	38 (28)	72 (16)	40 (23)	26 (22)	74 (12)	52 (18)	48 (22)
0.25% phytic acid (PA) ^c	62 (23)	40 (14)	18 (11)	63 (27)	46 (9)	39 (4)	58 (23)	35 (13)	27 (8)
1% β -CD ^d (8.8 mM)	74 (16)	29 (10)	-1 (8)	80 (16)	31 (15)	11 (19)	78 (17)	33 (13)	13 (8)
+0.5% SAPP ^a	106 (4)	105 (4)	153 (26)	96 (2)	94 (2)	98 (5)	102 (2)	102 (2)	111 (6)
+0.5% SHMP ^c	105 (2)	101 (4)	128 (14)	87 (9)	82 (11)	76 (17)	104 (3)	102 (2)	103 (4)
+0.25% Sporix ^c	99 (15)	93 (14)	138 (45)	84 (8)	85 (16)	94 (9)	102 (6)	99 (6)	108 (7)
+0.25% PA ^e	102 (4)	106 (4)	120 (14)	98 (5)	97 (4)	92 (8)	104 (4)	105 (6)	110 (14)
1% maltosyl β -CD ^d (~7 mM)	76 (14)	33 (10)	0 (9)	76 (18)	30 (13)	2 (15)	79 (18)	38 (14)	12 (12)
+0.25% Sporix ^f	98 (0)	100 (0)	124 (8)	60 (16)	72 (12)	80 (10)	109 (3)	106 (2)	112 (2)
4% maltosyl β -CD ^d (~28 mM)	140 (6)	124 (8)	73 (29)	134 (32)	106 (23)	31 (14)	106 (15)	99 (9)	60 (10)
+0.25% Sporix ^f	106 (2)	107 (1)	126 (4)	86 (1)	90 (1)	90 (4)	108 (4)	107 (2)	113 (2)

^a Four samples used in treatment study. ^b Values in parentheses are standard deviations of the mean. ^c Six samples used in treatment study. ^d From Table 1. ^e Ten samples used in treatment study. ^f Two samples used in treatment study.

tory properties similar to the latter compound when used at low (1%) concentrations (Table 1). Increasing the concentration of this more soluble derivative to 4% (~28 mM) led to a complete inhibition of browning for 6 h and substantial inhibition (60%) for 25 h at room temperature storage. Hydroxyethyl β -CD, another soluble derivative, gave similar results to the maltosyl derivative, and addition at 10% (~80 mM) concentration prevented browning from occurring completely for at least 4 days at room temperature and for over 1 year under refrigerated conditions (data not shown).

Effects of Soluble Cyclodextrins in Combination with Phosphates. Although high concentrations of β -CD derivatives were effective at preventing browning in apple juice, the use of such high levels in a food product may be impractical. Hence, we investigated the use of lower levels of CDs in conjunction with other inhibitors of enzymatic browning. Table 2 lists the browning inhibition resulting from the addition of low levels (either 0.5 or 0.25% wt/vol) of inorganic and organic phosphates in the apple juice model system. Each of the GRAS additives, SAPP and SHMP, were ineffective alone at preventing browning. Sporix, an acidic polyphosphate, and phytic acid, neither approved for food use, were slightly more effective, but this was probably due to a simple pH effect (see Sapers et al. 1989). The combination of only 1% β -CD with any of these four phosphate-containing compounds resulted in complete inhibition of browning for more than 25 h at room temperature and for some combinations, up to 2–3 weeks under refrigerated conditions (Figure 1). Comparison of % inhibition values in Table 2 for β -CD and each phosphate alone with that resulting from the combination treatment reveals a synergistic effect; the inhibition obtained by the combination treatment is almost always greater than the sum of the two single treatments. The combination of the 1% maltosyl β -CD with Sporix gave results similar to the combination of 1% regular β -CD with Sporix.

Use of Insoluble β -CD Polymers in a Semicontinuous Mode. Use of soluble cyclodextrins or their derivatives is approved for food applications in several countries around the world but not presently in the United States. For this reason, we examined the use of an insoluble β -CD polymer as a processing aid for the production of treated juices that would, presumably, contain no cyclodextrin in the final product. The resulting qualitative data (Table 3) indicates that treatment through a column of insoluble β -CD polymer inhibited enzymatic browning in juices from three

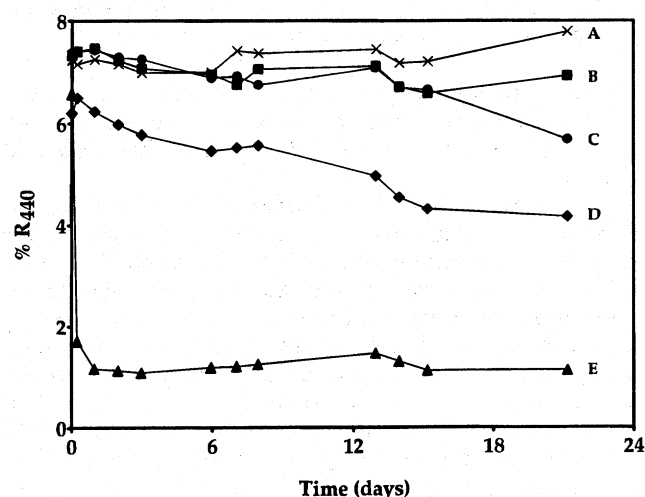


Figure 1. Percent reflectance values at 440 nm (% *R*₄₄₀) for raw Granny Smith apple juice containing 1% β -CD + A = 0.5% SAPP; B = 0.5% SHMP; C = 0.25% Sporix; D = 0.25% phytic acid; E = control juice. One typical experiment; juice samples were held at 4 °C.

Table 3. Inhibition of Enzymatic Browning Using Insoluble-CD Polymer in a Semicontinuous Process

fruit	sample	time before browning ^a
grapes	control	4 h
	treated	>72 h
apple	control	1 h
	treated	>82 h
pear	control	20 min
	treated	>60 h
celery	control	24 h
	treated	>48 h

^a Samples were held at room temperature and monitored visually.

different fruits and from celery. Whereas control samples browned within a few minutes to 24 h, treated samples did not undergo any visually detectable browning during the entire course of the experiment. The times given for the treated samples represent the time the experiment was ended due to visible microbial spoilage. All juices prepared by this method were crystal clear and contained none of the suspended solids normally found in raw juices. Presumably, this was because the packed column of polymer acted as an efficient filter, removing cell debris and particulate material as the sample passed through the bed. This suggests that browning inhibition may have been due in part to removal of particulate-bound PPO, as reported by Sapers (1991).

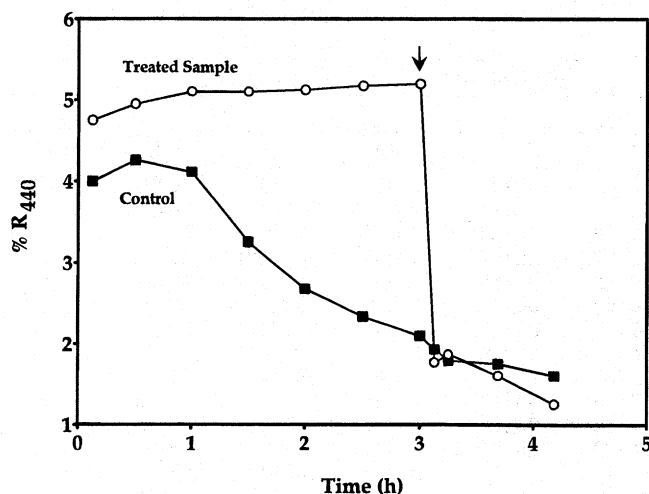


Figure 2. Percent reflectance values at 440 nm (% R_{440}) for control and insoluble β -CD polymer treated Granny Smith apple juice held at room temperature. An arrow represents the addition of chlorogenic acid to treated sample.

Although such juices may have useful applications, the following treatment was devised to produce a product that more closely resembled that of a freshly produced raw juice.

Use of Insoluble β -CD Polymer in a Batchwise Mode. Juice treated by this process had natural color and clarity indistinguishable from untreated, fresh raw juice. Reflectance measurements (Figure 2) were taken on treated and untreated juices. The % R_{440} values of these treated juices, held under refrigeration, remained unchanged for over 1 week (data not shown). Addition of chlorogenic acid, as shown in Figure 2, resulted in immediate browning, indicating that batchwise treated juices contain all necessary components for enzymatic browning (e.g., oxygen and PPO) except phenolic substrates.

Since no sensory data was performed here, it is not known if essential flavor, aroma components, or natural pigments were also removed by the CD treatment. However, it has been shown that similar CD polymers removed the bitter components naringen and limonin from citrus juices without removing essential oils, flavors, and vitamins (Shaw et al., 1984; Shaw and Wilson, 1985). On the other hand some loss of esters and alcohols important to apple aroma might be expected (Ito et al., 1988). Complete compositional and sensory analyses of these treated juices remains to be done. Further research is also needed to optimize CD use in juice processing and to establish the economic feasibility of this technology. Such issues as the optimal ratio of insoluble β -CD polymer to juice; the enhancement of insoluble polymer performance by phosphate addition, as proved effective with the soluble CDs; the compatibility of CD treatment times with conventional juice production rates; and the ease of recovery and regeneration of spent CD polymer need to be examined.

In conclusion, cyclodextrins, both soluble and insoluble, alone or in combination with organic or inorganic phosphates, can be used to inhibit enzymatic browning in fresh, raw fruit and vegetable juice. Products based on these technologies that could meet regulatory and labeling requirements would represent a new concept in minimally-processed fruit beverages.

ACKNOWLEDGMENT

We thank D. Downing, A. Hedges, G. Reed, and R. Friedman from American Maize Products Co., P. Hereld

from the Hereld organization, and J. Hashimoto of Ensuiko Sugar Refining Co. for samples of cyclodextrins. We thank ARS, USDA, for providing C.B.S.T. an Administrator-funded Research Associate position.

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Received for review March 15, 1996. Revised manuscript received June 4, 1996. Accepted June 7, 1996.* Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

JF960181H